

Analyses of a Debilitating Parasite (*Microphallus papillorobustus*, Trematoda) and Its “Hitchhiker” Parasite (*Maritrema subdolum*, Trematoda) on Survival of Their Intermediate Host (*Gammarus insensibilis*, Amphipoda)

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ABSTRACT: Parasites that alter their intermediate host's behavior to favor its predation by the definitive host are known from a wide range of host–parasite associations. Recently, we found a new category of parasites, so-called “hitchhikers,” unable to modify the behavior of their intermediate host but exploiting the same host spectrum, that gain benefits in transmission success from the infection of these behaviorally manipulated hosts. Because the probability of successful transmission by “hitchhiking” depends on 1) the efficiency of the “favorization” process of the debilitating parasite and 2) the effects of infection by the hitchhiker parasite on the host survival, we investigated these two aspects. In the laboratory, we showed that, in the absence of a predator (definitive host), there is no significant difference between the mortality rates of *Gammarus insensibilis* (second intermediate host) uninfected and infected by the debilitating trematode *Microphallus papillorobustus*. In the field, we showed that the hitchhiker trematode *Maritrema subdolum* does not significantly reduce host survival. These results suggest that the higher mortality rate of manipulated hosts in the field could be explained by the predation by the definitive hosts and that *M. subdolum* does not alter, through survival reduction, the efficiency of the favorization process.

KEY WORDS: trematode, *Gammarus*, *Microphallus papillorobustus*, *Maritrema subdolum*, survival, hitchhiking strategy.

In a wide range of complex parasite life cycles, parasites have been shown to modify the behavior of their intermediate hosts to enhance their probability of predation by the definitive host (Bethel and Holmes, 1973; Camp and Hui-ting, 1979; Giles, 1983; Hoogenboom and Dijkstra, 1987; Barnard and Behnke, 1990; Combes, 1991; Esch and Fernandez, 1993; Poulin, 1995). Even if they are difficult to quantify, these host manipulations are probably often costly to achieve (Poulin, 1995). Theory predicts that other parasites would benefit from such efficient mechanisms of “favorization,” through preferentially infecting hosts previously infected by a debilitating parasite (Combes, 1991; Poulin, 1994; Lafferty and Morris, 1996). These parasites could obtain a high probability of transmission, making no investment in manipulation themselves. Recently, Thomas et al. (1997) provided possible evidence for such a “hitchhik-

ing” strategy from a complex association involving two trematode parasites and one gammarid species as intermediate host: gammarids (*Gammarus insensibilis*) infected by *Microphallus papillorobustus* Rankin, 1940 (Trematoda, Microphallidae), are named “mad gammarids” because they display an aberrant behavior that renders them more vulnerable than uninfected ones to predation by aquatic birds, the definitive hosts (Helluy, 1983a, 1983b); *Maritrema subdolum* Jägerskiöld, 1909 (Trematoda Microphallidae), enhances its transmission to the definitive hosts by parasitizing amphipods previously infected by the debilitating trematode (i.e., *M. papillorobustus*).

Infection by parasites tends frequently to reduce the energy available for sustaining essential functions and, consequently, may increase the host mortality rate (Kinne, 1984; Combes, 1995). The probability of successful transmission by hitchhiking depends on 1) the efficiency of the “favorization” process of the debilitating parasite, and 2) the effects of infection by the

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hitchhiker parasite on the host survival. Although the demographic impact of *M. papillorobustus* is considerable in field populations of *G. insensibilis* (Thomas et al., 1995a), we do not know whether or not predation by definitive hosts (i.e., Charadriiform birds) is the major cause of "mad gammarids" mortality. In this paper, we first analyze whether mad gammarids, compared with uninfected ones, display a reduced vigor that could enhance their mortality rate even in the absence of predators. Second, we analyze the precise influence of *M. subdolum* on the mortality of its gammarid host in the field.

Materials and Methods

Infection by *M. papillorobustus* and host mortality without predator

A large sample of unpaired *G. insensibilis* ($n = 209$) was collected during May 1995 in the Thau's lagoon (southern France, 43°25'N, 3°35'E), following the method described in Thomas et al. (1995a). We attempted to obtain equal numbers of infected and uninfected individuals. In the field, infected individuals were identified through the aberrant behavior induced by the parasite (Helluy, 1983a, 1983b; Thomas et al., 1995a). In the laboratory, gammarids were maintained individually in small cups (diameter: 2 cm, height: 5 cm) filled with constantly aerated seawater (20°C, 38‰), without food, and at the natural photoperiod (14 h light:10 h dark). The cups were examined daily, and dead individuals were immediately sexed, measured by length (from head to tip of telson), and dissected to count cerebral metacercariae of *M. papillorobustus*. Metacercariae of this trematode are ovoid cysts ($270 \times 350 \mu\text{m}$, Rebecq, 1964) located in the amphipod brain (Helluy, 1983a). We estimated prevalence (proportion of infected individuals, Margolis et al., 1982) and mean intensity of infection (mean parasite load of infected individuals) for both males and females. Since all individuals were entered simultaneously into the experiment and followed until death, we compared the survivorship between infected and uninfected males and females using a Kolmogorov-Smirnov test as recommended by Pyke and Thompson (1986).

Infection by *M. subdolum* and host mortality in the field

To determine whether metacercariae of *M. subdolum* induced host mortality, we collected a new sample ($n = 700$) of *G. insensibilis* at Thau's lagoon during March 1996. Because *M. subdolum* is found mainly in "mad gammarids" (Thomas et al., 1997), we collected only gammarids with an aberrant behavior, following the same methodology as before. Later, gammarids were sexed, measured in length, and dissected to count the number of *M. subdolum* metacercariae present. Metacercariae of *M. subdolum* are small cysts (diameter: $250 \mu\text{m}$, Rebecq, 1964) located in the abdomen (Helluy, 1981). Growth in gammarids conforms to a

logistic curve (Sutcliffe et al., 1981). Males and females were placed in 7 and 6 length classes, respectively (assuming there was a relation between age and size, Sutcliffe et al., 1981). In classes 2 to 6 for males, and 2 to 5 for females, steps were equal (i.e., 4 mm). Class 1 includes all individuals that were too small to be in class 2. Classes 7 for males, and 6 for females, include all individuals that were too large to be in classes 6 and 5, respectively. Following Anderson and Gordon (1982) and Rousset et al. (1996), we analyzed changes in mean parasite abundance of *M. subdolum* with host size.

Statistical tests were performed following Sokal and Rohlf (1981) and Siegel and Castellan (1988). Parametric statistics were used when appropriate; when conditions for their use were violated, equivalent non-parametric tests were used. All tests were two-tailed. Throughout the paper, values given are mean \pm SD. The significance level chosen was 5%. All analyses were performed using Logithec (V. Boy, Station biologique Tour du Valat).

Results

Infection by *M. papillorobustus* and host mortality without predator

The lengths of infected (I) and uninfected (U) individuals were not significantly different for males (I: $12.97 \text{ mm} \pm 3.22$, U: $12.85 \text{ mm} \pm 3.14$; ANOVA, $F_{1,111} = 0.03$, $P > 0.05$), or for females (I: $12.02 \text{ mm} \pm 2.95$, U: $11.73 \text{ mm} \pm 4.40$; Mann-Whitney test, $z = -1.7$, $P > 0.05$). The mean mortality date was unrelated to the size of individuals in both infected (males: $r = 0.23$, $n = 55$; females: $r = 0.08$, $n = 44$; $P > 0.05$ in both cases) and uninfected individuals (males: $r = -0.05$, $n = 58$; females: $r = -0.13$, $n = 52$; $P > 0.05$ in both cases).

Death mortality distributions are shown in Figure 1. There was no significant difference between uninfected and infected individuals for males (Kolmogorov-Smirnov two-sample test, $n_1 = 58$, $n_2 = 55$, $D = 0.13$, $P > 0.05$) or females (Kolmogorov-Smirnov two-sample test, $n_1 = 52$, $n_2 = 44$, $D = 0.10$, $P > 0.05$).

Among infected individuals, the mean intensity between males (2.56 ± 2.33) and females (2.27 ± 2.33) was not significantly different (Mann-Whitney test, $z = -0.2$, $P > 0.05$). There was no significant relationship between the date of mortality and the parasite intensity (Spearman rank order correlation, males $r_s = -0.18$; females $r_s = -0.03$; $P > 0.05$ in both cases).

Infection by *M. subdolum* and host mortality in the field

Mean sizes of males and females were $18.8 \text{ mm} \pm 6.6$ and $14.9 \text{ mm} \pm 5.3$, respectively. As

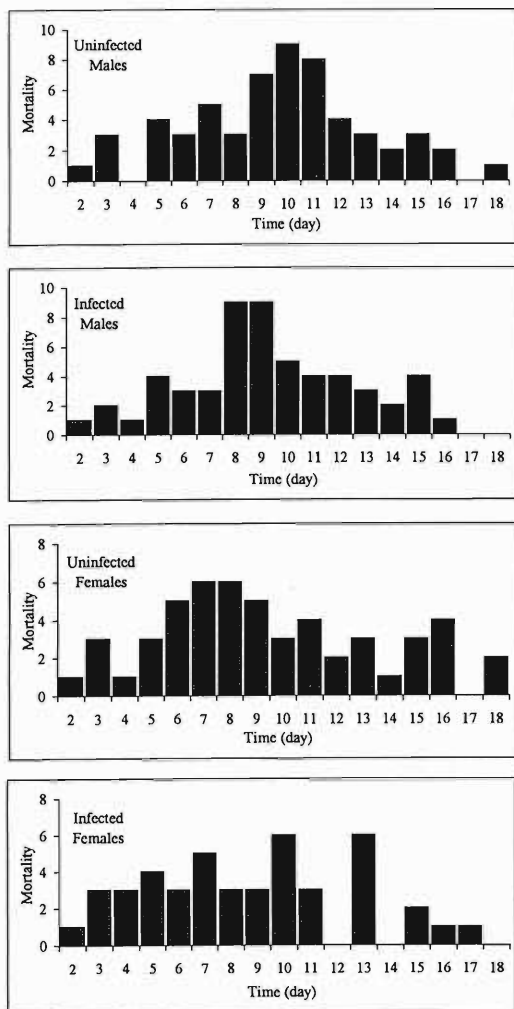


Figure 1. Mortality distributions (under starving condition) of *Gammarus insensibilis* uninfected and infected with metacercariae of *Microphallus papillorobustus*.

expected, all individuals were infected with cerebral metacercariae of *M. papillorobustus*. The mean intensities of *M. papillorobustus* for males were 4.5 ± 4.3 , and for females, 3.8 ± 3.5 , which is not significantly different (Mann-Whitney test, $z = -0.07$, $P > 0.05$). Prevalences of *M. subdolum* were 44% for males and 41% for females (Fisher's exact test, $P = 0.68$). Mean abundance in *M. subdolum* for males were 2.34 ± 1.4 , and 1.7 ± 1 for females (Mann-Whitney test, $z = -0.03$, $P > 0.05$), and mean intensities were 3.1 ± 2.6 and 2.4 ± 2 , respectively (Mann-Whitney test, $z = -1.9$, $P > 0.05$). For both males and females, fits on mean abundance with size were better from a nonlinear regression than a simple linear regression (males, $r^2 = 0.57$; females, $r^2 = 0.77$, Fig. 2). In males, a maximum mean abundance was not observed for larger hosts, but this effect was slight and was not supported in females, since the fitted polynomial curves did not show any decrease with host size. Relationships between variance to mean abundance ratio and size were not informative.

Discussion

Studies on parasites that enhance their hosts' susceptibility to predation by definitive hosts have not provided much quantitative field data to date. Consequently, the demographic impact of such parasitism and the main causes of mortality of manipulated hosts in the field are unknown. Infected gammarids do not differ from uninfected ones in their capacity to resist starvation. Assuming there is a close relationship between resistance to starvation and physical condition, this suggests that the parasite load of *M. papillorobustus* does not reduce significantly the energy available for sustaining essential

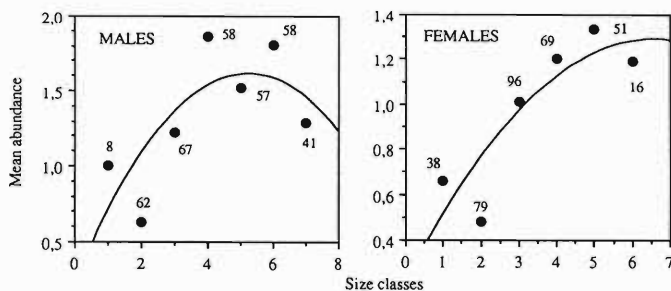


Figure 2. Changes in the mean abundance of *Maritrema subdolum* with host size in males and in females of *Gammarus insensibilis*. The number of hosts analyzed in each length class is indicated with each data point.

functions. Thus, in the absence of predators (i.e., definitive hosts), *M. papillorobustus* seems to have no significant detrimental effect on its hosts' survival. This situation contrasts with the results obtained by Helluy (1984) from experimental tests using a definitive host (i.e., *Larus cachinans*). Indeed, experimentally, the differential predation of infected gammarids considerably reduced their survival compared with uninfected ones. The present study suggests that the strong demographic impact of *M. papillorobustus* on *G. insensibilis* populations (Thomas et al., 1995a) is unlikely to be explained by a reduced vigor of infected hosts compared with uninfected ones. Unless this impact is the result of the detrimental effect of *M. papillorobustus* on the amphipod growth (Thomas et al., 1996) or of another, unidentified cause of mortality, it could be explained by the predation by definitive hosts.

The results obtained on survival in starving conditions are also informative in the context of sexual selection. Indeed, in the mating system of many amphipod species, males guard females for several days until the fertilization of eggs is possible (i.e., precopulatory mate guarding, see Crespi, 1989, for a review). For males, mating success and ability to guard females are correlated positively (Ward, 1983, 1984; Sutcliffe, 1993). This period is costly for males in terms of reduced opportunity to feed, since their gnathopods are occupied in holding females (Robinson and Doyle, 1985; Sutcliffe, 1993). Our results suggest that infected males, compared with uninfected ones, are not limited by their ability to resist starvation when they hold a female.

Compared with the results obtained with *M. papillorobustus* (Thomas et al., 1995a), *M. subdolum* has much less influence on the host population. Indeed, when the rate of host mortality is correlated positively with parasite accumulation (Anderson and Gordon, 1982; Rousset et al., 1996) or parasite presence (Rousset et al., 1996), curves of the parasite abundance as a function of the host age are convex and peak at much smaller host sizes, as a consequence of the deaths of the oldest infected hosts. The absence of effect may come from the fact that *M. subdolum* does not accumulate enough to significantly affect its host's survival. Nevertheless, similar results have been obtained for two other species of Microphallidae (i.e., *Microphallus hoffmanni*, Thomas et al., 1995b, and *Levinseni-*

iella tridigitata, Thomas et al., 1995c) that, like *M. subdolum*, encyst in the abdomen of *G. insensibilis* and never alter the behavior. Thus, it appears that at least in the present case, *M. subdolum* seems not to be a costly passenger in its host.

Acknowledgments

This work has been supported by a grant from the Ministère de l'Environnement (France), Comité Ecologie et Gestion du Patrimoine Naturel.

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